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Further Evaluation of the SG Test Strip for Estimation of Urinary Osmolality[1])

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Summary: The mass densities of 2000 urines from children and 2000 urines from adults were determined with the new test strip, Multistix SG, and the results were compared with osmolality measurements. Normal urinary parameters were investigated for possible interference with the test strip reading. Variations in the pH and ionic composition of urine considerably influenced the test strip reading, whereas nonionic compounds, such as glucose and urea, showed practically no interference. For patients on a normal diet and without metabolic diseases, the new test strip is more appropriate than urometry for the determination of the mass density of the urine.

Weitergehende Untersuchungen zur Abschätzung der Urin-Osmolalität mit dem SG-Teststreifen

Zusammenfassung: Bei 2000 Urinen von Kindern und bei 2000 Urinen von Erwachsenen wurde die Dichte des Urins durch den neuen Teststreifen Multistix SG bestimmt und mit den Ergebnissen der Osmometrie verglichen. Interferenzen der Teststreifenanzeige durch physiologische Urinbestandteile wurden ausführlich untersucht. Es ergab sich ein erheblicher Einfluß des Urin-pH und der ionalen Zusammensetzung auf das Teststreifenergebnis. Dagegen interferierten nichtionische Bestandteile wie Glucose und Harnstoff praktisch nicht. Die Dichte des Urins bei Patienten mit üblicher gemischter Kost und ohne Störungen im Säuren-Basen-Haushalt kann mit dem neuen Teststreifen besser untersucht werden als mit der Urometrie.

Introduction

The urinary test strip Multistix SG includes a newly developed test field ("SG" stands for specific gravity = mass density), which is useful for the estimation of urinary mass density. With regard to the reaction principle used (measurement of the ionic concentration of strong cations) this field rather gives a description of the osmolal activity of the urine. Recently we published our initial results on the empirical correlation of the test strip indication (SG) and urinary osmolality (1). In the meantime, contradictory results (2–8), based on a rather small number of urine samples, have been published. We now report a comprehensive evaluation, which defines more precisely the feasibility and limitations of the SG field.

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Materials and Methods

Urinary test strip Multistix SG, Miles/Ames, Frankfurt.

The urine samples consisted of 2000 urine specimen from adults (in-patients of the 1st Clinic, Medical School of the University of Kiel) and 2000 urine specimen from children of different ages (in- and out-patients of the Children's Hospital, Medical School of the University of Kiel). All urine specimens from adults were sampled in the morning, those from children being sampled in the morning and before noon. Test strip examination and determination of osmolality were not performed later than 4–5 hours after sampling. An interpolation of test strip reading was recommended for the evaluators.

The results were registered, together with the pH value of the urine and the concentrations of glucose, protein and ketone bodies; blood, if present, was also determined. According to the recommendation of the manufacturer, the test strip result was adjusted to higher values by adding 0.005 (corresponding to 1 field) in all urines with a pH $\geq$ 6.5. All tests were performed by experienced technicians of the clinical laboratory. All samples with an obvious discrepancy between the test strip result and osmolality were kept frozen for further examination.

Urines that gave results outside the 80% range (68 adults, 96 children), and other urines showing values within the 80% range (76 adults, 101 children) (cf. results) were examined in more detail: pH (glass electrode), sodium and potassium (flame photometry), calcium and magnesium (atomic absorption spectrophotometry), chloride (coulometry), phosphate (ammonium molydate method), urea (acc. to *Berthelot*), uric acid (fully enzymatic aldehyde dehydrogenase method) and creatinine (*Jaffé*, kinetic assay). All routine methods were used under quality control supervision. Ammonia was determined according to *Berthelot*.

Interferences by certain cations and anions were examined in binary systems. For this purpose isoosmolal solutions of sodium chloride and the salt to be examined were made with approximately the same pH. These two solutions were mixed in dilution series: 10 + 0, 9.5 + 0.5, 9 + 1, 8 + 2, . . . etc.

Osmolality and test strip reaction were measured in duplicate.

Results

Correlations of SG test strip reading and osmolality in children and adults

The SG test field yields dubious results in urines with a pH $\geq$ 8.0, as will be shown later. Furthermore the SG field does not respond to glucose. Thus all urines with a pH $\geq$ 8.0 and a glucose concentration $\geq$ 1 g/l were omitted from tables 1–3 and figures 1–2, which compare urinary osmolalities with the various classes of test strip response. These tables and figures include results for a total of 1815 children's and 1824 adults' urines.

To examine the correlation between SG test strip reading and osmolality a non-parametric and graphical approach was chosen. In the graphic presentation of figures 1 and 2 the osmolality percentile values P_{10}, P_{50} and P_{90} of each test strip class are connected.

Account was taken in the graphic approximation of the fact that some classes were less frequently represented (e.g. 1.0175 and 1.0225 kg/l) than the main classes. The correlation lines show a sigmoidal shape in children, and a linear correlation in adults.

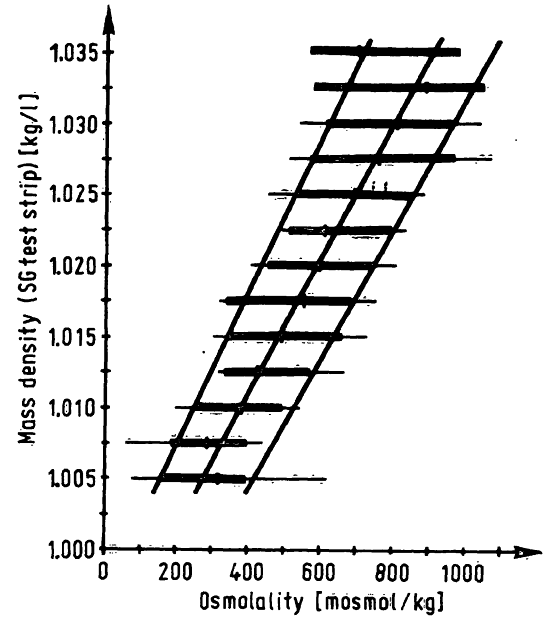

Fig. 1. Correlation of SG test strip reading and osmolality in adults. P_5, P_{10}, P_{50}, P_{90}, and P_{95} of osmolality in the various test strip classes are given.
P_{10-90}; —— P_{5-95}

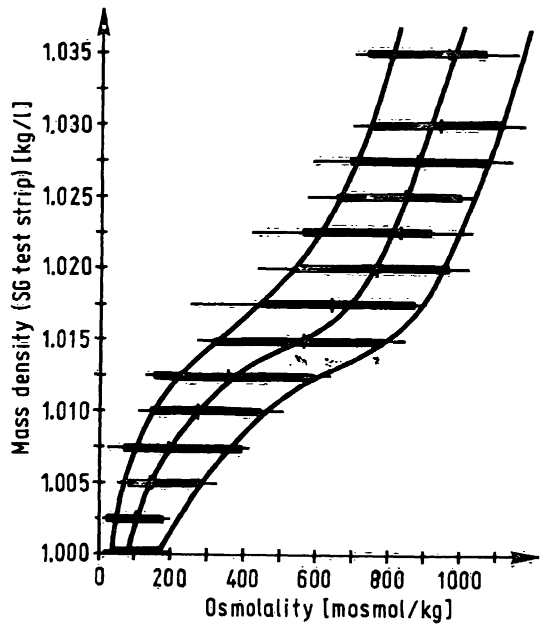

Fig. 2. Correlation of SG test strip reading and osmolality in children. P_5, P_{10}, P_{50}, P_{90} and P_{95} of osmolality in the various test strip classes are given.
P_{10-90}; —— P_{5-95}

There are obvious differences between the results seen in children and adults. Children's urines more often have osmolalities below 400 mosmol/kg and these urines render a higher test strip reading than comparable urines of adults. In the range of medium urine concentration (SG test strip reading 1.015 and

Tab. 1. Percentiles of osmolalities measured in the different SG test strip reading classes 1.000–1.035. Corresponding values of adults and children are given.

SG reading (kg/l)	Number of children/adults	Osmolality [mosmol/kg] $P_{2.0}$	P_5	P_{10}	P_{25}	P_{50} (Median)	P_{75}	P_{90}	P_{95}	$P_{97.5}$
1.000	25/0	–/–	8/–	34/–	55/–	83/–	122/–	156/–	168/–	–/–
1.002	18/1	–/–	11/–	19/–	83/–	101/64	155/–	179/–	194/–	–/–
1.005	130/20	36/78	51/78	79/161	106/216	144/318	200/364	284/397	324/623	510/670
1.007	49/22	13/65	22/65	68/185	115/253	192/289	307/344	396/397	416/442	438/442
1.010	217/232	93/192	108/200	141/248	206/307	272/381	355/444	449/500	510/545	632/593
1.012	53/81	108/248	127/319	149/333	245/387	355/428	455/511	595/577	641/669	681/808
1.015	278/427	244/266	270/305	318/345	403/398	564/496	693/584	795/662	848/730	906/770
1.017	47/70	174/303	251/323	440/338	488/468	640/531	807/613	876/689	904/760	929/771
1.020	258/296	356/362	438/411	487/455	629/529	765/600	880/685	970/760	1025/814	1037/845
1.022	29/26	420/497	420/497	558/512	617/545	830/612	879/686	920/802	1036/842	1036/842
1.025	298/289	536/418	575/459	652/543	745/622	843/695	936/781	1002/860	1046/894	1046/948
1.027	36/46	362/511	592/517	689/576	793/687	878/761	963/892	1080/976	1147/1078	1233/1148
1.030	354/300	656/514	701/545	758/617	844/708	940/813	1050/906	1123/976	1180/1045	1261/1116
1.032	1/8	–/–	–/–	–/584	–/779	750/893	–/961	–/1009	–/–	–/–
1.035	22/6	–/–	706/–	738/571	813/639	962/706	1005/989	1071/989	1112/–	–/–

Tab. 2. Percent frequency of osmolality results (7 classes) in the various test strip reading classes: adults' data.

SG reading (kg/l)	n	Frequency (%) in osmolality (mosmol/kg) classes –200	–400	–600	–800	–1000	–1200	–1400
1.035	6	–	–	16.7	33.3	33.3	16.7	–
1.0325	8	–	–	12.5	12.5	50.0	25.0	–
1.030	300	–	0.7	8.3	38.3	44.3	8.3	–
1.0275	46	–	–	13.0	45.7	30.4	6.5	4.3
1.025	289	–	2.1	20.1	57.1	19.7	1.0	–
1.0225	26	–	–	46.2	38.5	11.5	3.8	–
1.020	296	–	4.4	46.3	42.6	6.4	0.3	–
1.0175	70	–	12.9	58.6	25.7	2.9	–	–
1.015	427	0.2	25.5	52.0	20.8	1.2	0.2	–
1.0125	81	–	32.1	59.3	4.9	3.7	–	–
1.010	232	5.2	55.6	36.6	2.2	0.4	–	–
1.0075	22	9.1	81.8	9.1	–	–	–	–
1.005	20	15.0	75.0	–	10.0	–	–	–
1.0025	1	100	–	–	–	–	–	–
1.000	0	–	–	–	–	–	–	–

Tab. 3. Percent frequency of osmolality results (7 classes) in the various test strip reading classes: children's data.

SG reading (kg/l)	n	Frequency (%) in osmolality (mosmol/kg) classes –200	–400	–600	–800	–1000	–1200	–1400
1.035	22	–	–	–	22.7	45.5	31.8	–
1.0325	1	–	–	–	100	–	–	–
1.030	354	0.3	–	1.1	13.3	48.3	33.1	4.0
1.0275	36	–	2.8	2.8	2.0	55.6	13.9	0.6
1.025	298	–	0.7	5.4	31.5	51.7	9.7	1.0
1.0225	29	–	–	17.2	24.1	51.7	6.9	–
1.020	258	–	3.5	15.9	38.4	34.9	7.4	–
1.0175	47	2.1	6.4	31.9	27.7	31.9	–	–
1.015	278	1.1	23.4	37.1	28.8	9.0	0.7	–
1.0125	53	17.0	45.3	30.2	7.5	–	–	–
1.010	217	22.6	57.6	16.6	2.8	–	0.5	–
1.0075	49	53.1	36.7	10.2	–	–	–	–
1.005	13	75.4	20.8	1.5	2.3	–	–	–
1.0025	18	100	–	–	–	–	–	–
1.000	25	100	–	–	–	–	–	–

1.020 kg/l) corresponding percentiles of osmolality are higher in children than in adults and they cover a broader range. Studying the age-specific differences of children's samples the well known fact that most urines of newborns and infants are found in the SG test strip reading range ≤ 1.015 kg/l and those of older children in the SG test strip reading classes ≥ 1.015 kg/l was confirmed. But an age dependency of the correlation of SG test strip reading and osmolality can not be derived.

The urines of children and adults were tested simultaneously by the same technicians and the same methods. Therefore the different results in children's and adults' urine specimens are due to their different compositions. The urines which we examined in more detail showed a higher total anion related phosphate excretion. Furthermore the mean quotient

$$\frac{\Sigma\ [NH_4^+] + [Na^+] + [K^+]}{\text{osmolality}}$$

is higher in children than in adults. This is especially evident in the low SG test strip reading classes.

Finally the urines of children have significantly higher pH values than those of adults with the exception of extreme SG test strip reading classes (fig. 3).

The effects of pH, concentration of cations and the phosphate concentration (both relative to osmolality) may have an additive effect, resulting in different correlations of SG test strip reading and osmolality in children and adults.

At the very beginning of this study it was noticed that strongly alkaline urines showed extremely low SG test strip readings in comparison with the osmolality measured. Our large number of data confirms the strong influence of urine pH values ≥ 8.0 on the SG test strip reading. Urine values derived from samples with pH $\simeq 5$ are equally distributed around the P_{50} osmolality values of each corresponding test strip class, i.e. these urines present neither SG field enhancement nor depression.

In those few cases where the urine pH value was *below* 5, the SG test strip reading was always too high.

The manufacturer of the SG strip recommends a correction of the readings for alkaline urines (c.f. methods). To evaluate this correction we compared the pH of samples with the osmolality in *single SG test strip reading classes.*

Figure 4 for example shows the urinary osmolalities of the children's samples with SG test strip reading = 1.015 kg/l. These samples are classified according to

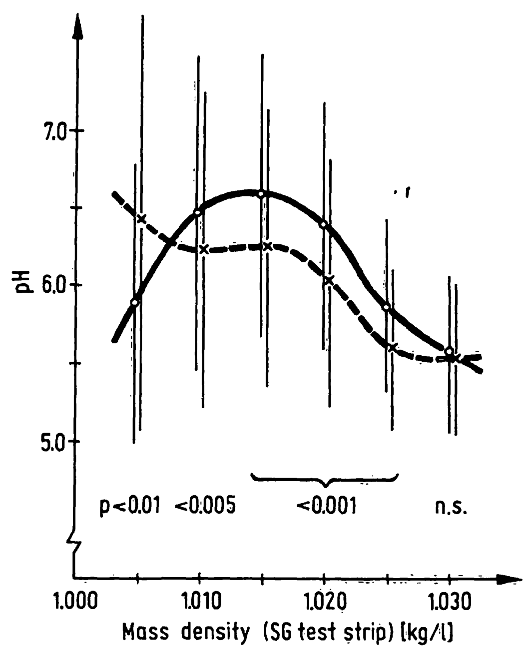

Fig. 3. Mean and standard deviation of urinary pH of children (O) and adults (x) in various SG test strip reading classes. Statistical significant differences between children and adults were found by *Student*'s t-test (n.s. = not significant).

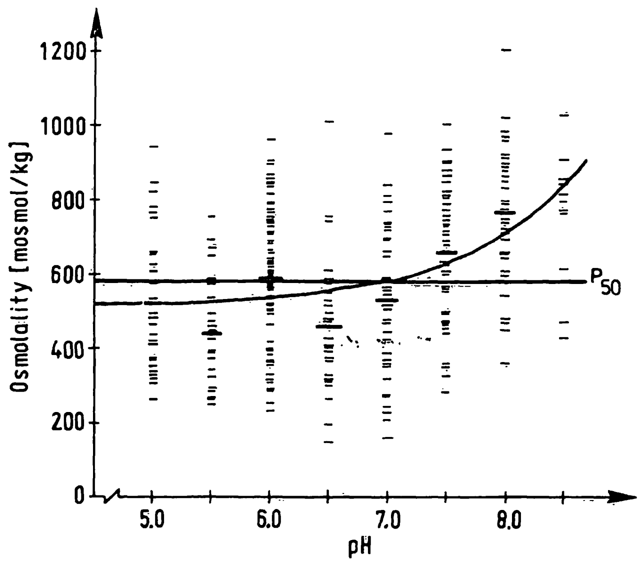

Fig. 4. Distribution of osmolality values in the SG test strip reading class 1.015 (children) in dependency on the urinary pH. The short lines show the individual values, the stronger lines the arithmetic means and the long line the P_{50} value of all values.
$y = 512.8 + 0.06643 \cdot e^x$

their pH. Figure 4 gives the osmolalities in these subclasses, their mean value and the mean of the total group (587 mosmol/kg). It may be seen that

a) urines with a pH ≥ 8.0 are in the wrong class, in spite of the correction, as their mean is rather high.

b) urines with pH of 7.0 and 7.5 are "correctly" classified

c) most urines with a pH of 6.5 may enter a falsely high class due to the correction, i.e. the correction is unnecessary.

Influence of the ionic composition of urine on the SG test strip reading

The test principle of the SG test field consists of release of H^+-ions from a polymer by strong cations followed by detection of H^+ by a pH indicator. This H^+-release is proportional to the osmolal activity of cations in the urine.

Obviously this procedure is enhanced by the anions phosphate and citrate, and the cations NH_4^+, Ca^{2+} and Mg^{2+} are more effective than Na^+ and K^+. This is demonstrated by figure 5 for NH_4^+ and phosphate. If sodium chloride ("A") and ammonium chloride ("B") solutions of isoosmolal activity (300 mosmol/kg) are mixed in increasing ratios as shown in the right part of figure 5, the resulting osmolality is constant, but there is a minimal increase in SG test strip reading proportional to NH_4^+ concentration. Ca^{2+} and Mg^{2+} show an analogous effect, K^+ does not. Phosphate, in contrast to chloride, enhances the SG test strip reading (upper part of fig. 5):

NaCl, volume fraction	NaH_2PO_4, volume fraction	SG test strip reading, kg/l
1.00	0	1.005
0.75	0.25	1.015
0.50	0.50	1.025
0.25	0.75	1.028
0	1.00	1.030

Figure 5 demonstrates the relations in a quaternary system of NaCl, NaH_2PO_4, NH_4Cl and $NH_4H_2PO_4$. This figure enables a prediction of the SG test strip reading in any mixture of these four salt solutions.

The sum of the straight lines $\overline{B'X} + \overline{A'X} + \overline{C'X} + \overline{C'X}$ may be designated as Σ.

The mixture at point X contains $\frac{\overline{B'X}}{\Sigma}$ parts B + $\frac{\overline{A'X}}{\Sigma}$ parts A + $\frac{\overline{C'X}}{\Sigma}$ parts C + $\frac{\overline{D'X}}{\Sigma}$ parts D.

The SG test strip reading at this point is 1.015 μg/l, the osmolality of the whole system 300 mosmol/kg.

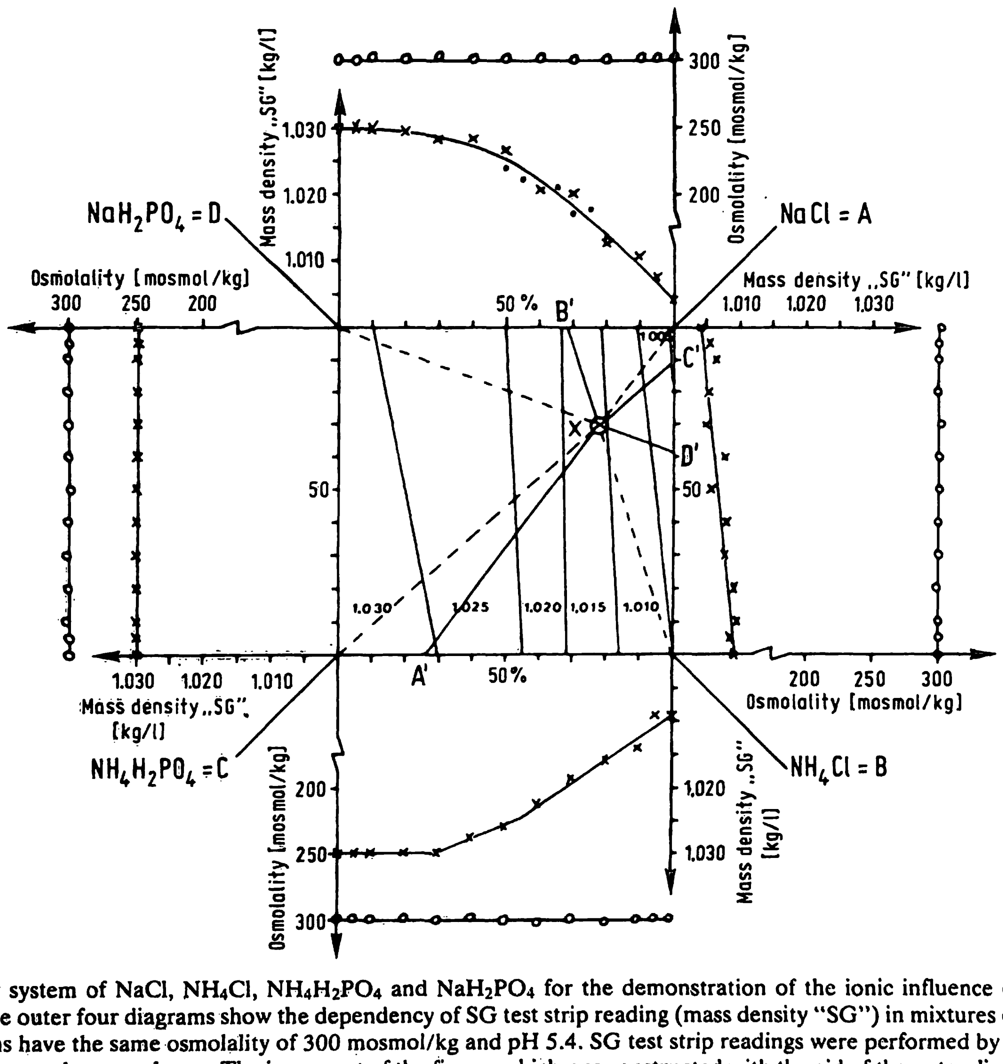

Fig. 5. Quaternary system of NaCl, NH_4Cl, $NH_4H_2PO_4$ and NaH_2PO_4 for the demonstration of the ionic influence on SG test strip reading. The outer four diagrams show the dependency of SG test strip reading (mass density "SG") in mixtures of two solutions. All solutions have the same osmolality of 300 mosmol/kg and pH 5.4. SG test strip readings were performed by several persons, and their mean values are shown. The inner part of the figure, which was constructed with the aid of the outer diagrams, allows an estimation of SG test strip reading in mixtures of these four solutions (c.f. results).

In an analogous way the strong enhancement of citrate was proven. Acetate, lactate, urate, oxalate and sulphate caused no deviations compared with the results obtained with chloride. Hydrogen carbonate could not be examined, because of the erroneous SG test strip readings at pH 8.3 ($NaHCO_3$).

Influence of the nonionic composition of urine on the SG test strip reading

Nonionic constituents of urine, such as glucose, urea and creatinine, do not directly affect the SG test field; the concentrations of these compounds exert an empirical influence on the correlation, mass density/osmolality.

Thus, urines with highly abnormal concentrations of these substances (relative to total osmolality) give "false" SG test strip readings. A concentration of 1.8 g/l *glucose* equals 10 mosmol/kg, and 18 g/l glucose equals 100 mosmol/kg. From the urinary osmolalities of table 1, it is obvious that only a very strong glucosuria (detectable by the glucose field of Multistix SG), together with a low osmolality, causes wrong SG test field readings.

In the samples submitted to more detailed study, the proportion of urinary osmolality due to *urea* varied between 20 and 60%. In very few cases extremly low or high urea concentrations were accompanied by inaccurate SG test strip readings.

The urinary *creatinine* concentrations are too low to influence the SG test strip reading.

Protein concentrations ≥ 1 g/l cause a tendency to higher SG test strip readings, yet nearly all results were in the 80% range of the osmolalities in the corresponding test strip classes.

In contrast to protein, *ketone bodies* cause lower SG test strip readings, but these also were mostly within the 80% range.

Discussion

The new SG test field was developed for the semiquantitive estimation of urinary mass density. The reference method used was refractometry of total solids, a method which is seldom used in Europe. Several factors support the principle of measuring the performance of the SG test field by osmolality rather than by refractometry:

1) The physiologically important parameter for the assessment of renal concentrating ability is the osmotic pressure of the urine.
2) Urometry and refractometry are imprecise methods susceptible to interferences.
3) The test principle of the SG test field resembles more closely osmometry than urometry or refractometry.

The correlation of SG test strip reading and osmometry (tab. 1) is worse than that for any other semiquantitative test strip and a corresponding quantitative measurement. The 90% range of osmolalities in children is even broader than in adults. Other authors (4) reported similar results. We would like to stress two points:

1) Our unselected material was received from patients of all ages, with different diagnoses and various forms of therapy. It was a classical field study.
2) The SG test field reacts with 1/3 of the osmotically active urinary constituents, the others contributing only empirically to the SG test strip reading.

We do not know exactly which diseases and which nutritional effects influence this empirical correlation. It may well be possible that more homogeneous random samples would result in a more defined 90% range.

Interference by several urinary constituents has been shown. Strongly alkaline and strongly acidic urines (pH > 8.0 and < 4.9) are not suitable for readings with the SG test field. Urines of patients with bacterial urinary tract infections, with severe disturbances of acid base balance and possibly with extreme nutritional habits (rich in vegetables: alkaline urine, rich in proteins, in particular meat: acid urine) should not be examined by the SG test strip.

Strongly alkaline urine may easily be recognized by the pH test field which is part of the Multistix SG. Thus the number of samples suitable for Multistix SG testing is decreased. We do not feel that this is a substantial limititation of efficacy.

Concerning the pH-corrected SG test strip readings in urine ≥ 6.5, which is recommended by the manufacturer, this seems unnecessary, provided that all urines ≥ 8.0 are no longer tested.

Phosphate anions have a strong enhancing effect on SG test strip reading as shown in figure 5. In our experience, high phosphate concentrations mostly parallel high osmolality, i.e. the empirical correlation of SG test strip reading and osmolality is maintained.

On a diet composed mainly of milk and vegetables, in patients with hyperparathyreoidism and in general in children a relatively high urinary phosphate concentration may be found, resulting in a falsely elevated SG test strip reading. Of all the anions tested, only citrate resembles phosphate (cf. results) in causing a disproportionally high SG test strip reading. Normally the urinary citrate concentration is much lower than the phosphate concentration. In alkaline urines, however, the output may be 20–30 g/day (= 100 mmol/d) (9). The influences of alkaline urine and high citrate excretion on SG test strip reading are adverse and perhaps may neutralize each other.

Nonionic urinary constituents present in relevant concentrations are glucose and urea. Neither of these need be responsible for actual interference because strong glucosuria is easily detected by the glucose test field, and high urea concentrations are mostly found in urines with high alkali ion concentrations.

In a few cases, a diet rich in protein and poor in salt (3) may yield wrong SG test strip results.

The different findings in children and adults are not fully explained by our results. The differences in urinary pH values, relative phosphate concentrations and other unknown factors may be interrelated with each other.

A final assessment of the SG test field is not easy. It can never be characterized as a semiquantitative test for urinary osmolality. The manufacturer has outlined this product for estimation of urinary mass density. We did not compare the SG test field with urometry and we do not feel that this is worthwhile. In view of the well known systematic faults in daily ward work, the practicability of a test strip represents an enrichment of the diagnostic spectrum of urinary analysis for those patients with "normal" diet and "normal" metabolic condition. Preliminary results furthermore show that daily monitoring of intensive care patients may well be done within a constant and narrow range of error. Finally the SG test field integrated into a multi-field test strip opens a new dimension in semiquantitive urine analysis, because the content of glucose and protein, for example, should not be considered without additional information on the concentration of the tested urine.

Acknowledgement

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